Complete Set of Amended Claims

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2. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein and an affinity peptide tag consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) combining the sample containing the fusion protein with metal-chelate affinity particles suitable for binding said fusion protein, said metal-chelate affinity particles being insoluble in the sample;

(b) collecting the metal-chelate affinity particles;

(c) separating the metal-chelate affinity particles from the unbound remainder of the sample;

(d) optionally, resuspending the metal-chelate affinity particles in a solution;

(e) optionally, eluting said fusion protein from the metal-chelate affinity particles, followed by separating the metal-chelate affinity particles from said eluted fusion protein;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of 0.0005% - 2% (v/v) detergent sufficient to reduce loss of metal-chelate affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

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3. (amended) The method according to Claim 2, wherein the combining step (a) is carried out in the absence of detergent, but detergent is added prior to the collecting step (b).

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8. (amended) The method according to Claim 2, wherein said affinity peptide tag is six consecutive histidine residues.

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13. (amended) The method according to Claim 2, wherein said metal-chelate affinity particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

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14. (twice amended) The method according to Claim 2, wherein said metal-chelate affinity particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, and combinations thereof.

17. (amended) The method according to Claim 2, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.

18. The method according to Claim 17, wherein said nonionic detergent is selected from the group consisting of polyoxyethylene (10) cetyl alcohol, polyoxyethylene (20) cetyl alcohol, polyoxyethylene (23) lauryl alcohol, polyoxyethylene (4-5) *p-t*-octyl phenol, polyoxyethylene (7-8) *p-t*-octyl phenol, polyoxyethylene (9) *p-t*-octyl phenol, polyoxyethylene (9-10) *p-t*-octyl phenol, polyoxyethylene (9-10) nonylphenol, polyoxyethylene (20) sorbitol monolaurate, polyoxyethylene (20) sorbitol monopalmitate, polyoxyethylene (20) sorbitol monooleate, octyl-β-glucoside, APO-10, APO-12, cyclohexyl-*n*-ethyl-β-D-maltoside, cyclohexyl-*n*-hexyl-β-D-maltoside, cyclohexyl-*n*-methyl-β-maltoside, *n*-decanoylsucrose, *n*-decyl-β-D-glucopyranoside, *n*-decyl-β-maltopyranoside, *n*-decyl-β-D-thiomaltoside, *n*-dodecanoyl sucrose, and heptane-1,2,3-triol, and combinations thereof.

19. (twice amended) The method according to Claim 17, wherein said nonionic detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

20. The method according to Claim 17, wherein said anionic detergent is selected from the group consisting of sodium dodecyl sulfate (SDS), sarkosyl, and combinations thereof.

21. The method Claim 17, wherein said zwitterionic detergent is 3-[(cholamido-propyl)-dimethyl-ammonio]-1-propanesulfonate.

22. The method according to Claim 17, wherein said cationic detergent dodecyl-trimethyl ammonium chloride.

23. The method according to Claim 2, wherein the detergent, where present, is a nonionic detergent at a concentration of at least about 0.005% (v/v).

24. The method according to Claim 2, wherein the detergent, where present, is a nonionic detergent at a concentration not exceeding about 2% (v/v).

25. The method according to Claim 2, wherein the detergent, where present, is an anionic detergent at

a concentration of at least about 0.05% (v/v).

15 ~~26.~~ The method according to Claim ~~2~~ 1, wherein the detergent, where present, is an anionic detergent at a concentration not exceeding about 1% (v/v).

16 ~~27.~~ The method according to Claim ~~2~~ 1, wherein the detergent, where present, is a cationic detergent at a concentration of at least about 0.5% (v/v).

17 ~~28.~~ The method according to Claim ~~2~~ 1, wherein the detergent, where present, is a cationic detergent at a concentration not exceeding about 1% (v/v).

18 ~~29.~~ The method according to Claim ~~2~~ 1, wherein the detergent, where present, is a zwitterionic detergent at a concentration of at least about 0.01% (v/v).

19 ~~30.~~ The method according to Claim ~~2~~ 1, wherein the detergent, where present, is a zwitterionic detergent at a concentration not exceeding about 2% (v/v).

20 ~~34.~~ (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein molecule and an affinity peptide tag consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of metal-chelate affinity particles and incubating said metal-chelate affinity particles in the presence of a detergent;

(b) combining the sample containing the fusion protein with metal-chelate affinity particles suitable for binding said fusion protein, said metal-chelate affinity particles being insoluble in the sample;

(c) collecting the metal-chelate affinity particles;

(d) separating the metal-chelate affinity particles from the unbound remainder of the sample;

(e) optionally, resuspending the metal-chelate affinity particles in a solution;

(f) optionally, eluting said fusion protein from the metal-chelate affinity particles, followed by separating the metal-chelate affinity particles from said eluted fusion protein;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of 0.0005% - 2% (v/v) detergent, wherein the use of detergent is sufficient to reduce loss of metal-chelate affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

21 ~~39.~~ (amended) The method according to Claim ~~34~~ 20, wherein said affinity peptide tag is six consecutive histidine residues.

22 ~~44.~~ (twice amended) The method according to Claim ~~34~~ 20, wherein said metal-chelate affinity particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

23 ~~45.~~ (twice amended) The method according to Claim ~~34~~ 20, wherein said metal-chelate affinity particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, and combinations thereof.

24 ~~48.~~ (amended) The method according to Claim ~~34~~ 20, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.

25 ~~49.~~ The method according to Claim 48, wherein said nonionic detergent is selected from the group consisting of polyoxyethylene (10) cetyl alcohol, polyoxyethylene (20) cetyl alcohol, polyoxyethylene (23) lauryl alcohol, polyoxyethylene (4-5) *p-t*-octyl phenol, polyoxyethylene (7-8) *p-t*-octyl phenol, polyoxyethylene (9) *p-t*-octyl phenol, polyoxyethylene (9-10) *p-t*-octyl phenol, polyoxyethylene (9-10) nonylphenol, polyoxyethylene (20) sorbitol monolaurate, polyoxyethylene (20) sorbitol monopalmitate, polyoxyethylene (20) sorbitol monooleate, octyl-β-glucoside, APO-10, APO-12, cyclohexyl-*n*-ethyl-β-D-maltoside, cyclohexyl-*n*-hexyl-β-D-maltoside, cyclohexyl-*n*-methyl-β-maltoside, *n*-decanoylsucrose, *n*-decyl-β-D-glucopyranoside, *n*-decyl-β-maltopyranoside, *n*-decyl-β-D-thiomaltoside, *n*-dodecanoyl sucrose, and heptane-1,2,3-triol, and combinations thereof.

26 ~~50.~~ The method according to Claim ~~49~~ 25, wherein said nonionic detergent is polyoxyethylene (20) sorbitol monolaurate.

27 ~~51.~~ The method according to Claim ~~48~~ 24, wherein said anionic detergent is selected from the group consisting of sodium dodecyl sulfate (SDS), sarkosyl, and combinations thereof.

28 52. The method Claim 48, wherein said zwitterionic detergent is 3-[(cholamido-propyl)-dimethyl-ammonio]-1-propanesulfonate.

29 53. The method according to Claim 48, wherein said cationic detergent dodecyl-trimethyl ammonium chloride.

30 54. The method according to Claim 34, wherein the detergent, where present, is a nonionic detergent at a concentration of at least about 0.005% (v/v).

31 55. The method according to Claim 34, wherein the detergent, where present, is a nonionic detergent at a concentration not exceeding about 2% (v/v).

32 56. The method according to Claim 34, wherein the detergent, where present, is an anionic detergent at a concentration of at least about 0.05% (v/v).

33 57. The method according to Claim 34, wherein the detergent, where present, is an anionic detergent at a concentration not exceeding about 1% (v/v).

34 58. The method according to Claim 34, wherein the detergent, where present, is a cationic detergent at a concentration of at least about 0.5% (v/v).

35 59. The method according to Claim 34, wherein the detergent, where present, is a cationic detergent at a concentration not exceeding about 1% (v/v).

36 60. The method according to Claim 34, wherein the detergent, where present, is a zwitterionic detergent at a concentration of at least about 0.01% (v/v).

37 61. The method according to Claim 34, wherein the detergent, where present, is a zwitterionic detergent at a concentration not exceeding about 2% (v/v).

38 62. The method according to Claim 34, wherein the molecule is a nucleic acid and the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

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63. The method according to Claim 34, wherein the molecule is a protein or peptide and the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

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64. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein molecule and an affinity peptide tag consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) combining the sample containing the fusion protein with metal-chelate, magnetic affinity particles suitable for binding said fusion protein, said metal-chelate, magnetic affinity particles being insoluble in the sample;

(b) applying a magnetic field to the vessel so as to attract and immobilize the metal-chelate, magnetic affinity particles;

(c) separating the unimmobilized remainder of the sample from the immobilized metal-chelate, magnetic affinity particles;

(d) optionally, resuspending the metal-chelate, magnetic affinity particles in a solution;

(e) optionally, eluting said fusion protein from the metal-chelate, magnetic affinity particles, followed by separating the metal-chelate, magnetic affinity particles from said eluted fusion protein;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of 0.0005% - 2% (v/v) detergent sufficient to reduce loss of metal-chelate, magnetic affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

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65. The method according to Claim 64, wherein the combining step (a) is carried out in the absence of detergent, but detergent is added prior to the application of a magnetic field in accordance with step (b).

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66. (twice amended) A method for isolating a fusion protein comprising a peptide, polypeptide, or protein and an affinity peptide tag consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of metal-chelate, magnetic affinity particles and incubating said metal-chelate, magnetic affinity particles in the presence of a detergent;

(b) combining the sample containing the fusion protein with said metal-chelate, magnetic affinity particles suitable for binding said fusion protein, said metal-chelate, magnetic affinity particles being insoluble in the sample;

(c) immobilizing the metal-chelate, magnetic affinity particles by applying a magnet to said vessel;

(d) separating the remainder of the sample from the immobilized metal-chelate, magnetic affinity particles;

(e) optionally, resuspending the metal-chelate, magnetic affinity particles in a solution;

(f) optionally, eluting said fusion protein from the metal-chelate, magnetic affinity particles, followed by separating the metal-chelate, magnetic affinity particles from said eluted fusion protein;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of 0.0005% - 2% (v/v) detergent, wherein the use of detergent is sufficient to reduce loss of metal-chelate, magnetic, affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

43 67. The method according to Claim ~~14~~ 5 or ~~45~~ 23, wherein the polyethylene polymer is a polyvinyl alcohol.

44 68. The method according to Claim ~~14~~ 5 or ~~45~~ 23, wherein the silicate is selected from the group consisting of calcium silicate, magnesium silicate, aluminum silicate, and combinations thereof.

45 69. The method according to Claim ~~14~~ 5 or ~~45~~ 23, wherein the metal oxide is selected from the group consisting of titanium oxide, tin oxide, and combinations thereof.

46 70. (new) The method for isolating a fusion protein according to any one of Claims ~~64-66~~ 40-42, wherein said fusion protein comprises a peptide, polypeptide, or protein and an affinity peptide tag consisting of six consecutive histidine residues and said metal-chelate, magnetic affinity particles are nickel-nitrilotriacetic acid agarose beads.